Potential of Pseudomonas Aeruginosa to Control Sclerotium Rolfsii Causing Stem Rot and Collar Rot Disease of Tomato

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Abstract—The aim of this study was to isolate and select the highest potential activities of bacteria from 7 provinces in northeastern region of Thailand against Sclerotium rolfsii. Thirteen of 329 isolates were screened as antagonistic bacteria to inhibit S. rolfsii by dual culture assay. High percentages of inhibition were found in three isolates of UD1EBa-2, KK1EBa-3 and KK11EBa-3 with 51.25%, 56.25% and 60.00%, respectively. The culture filtrate of each bacterium was prepared to test the mycelial growth inhibition of S. rolfsii. The results showed that the culture filtrate could inhibit mycelial growth with 100%. The most effective isolate KK11EBa-3 was characterized to be rod shape, gram negative and non spore forming. The colony on Nutrient agar was circular, smooth, translucent, flat elevation. Based on molecular sequencing data, it was identified as Pseudomonas aeruginosa.

Index Terms—Sclerotium Rolfsii, Biocontrol, Antagonistic bacteria, culture filtrate

I. INTRODUCTION

Tomato (Lycopersicon esculentum Mill.) is one of the food crops cultivated worldwide. The major problem of tomato cultivation is stem rot and collar rot disease caused by a soilborne fungus, Sclerotium rolfsii. This disease devastates tomato growing regions and causes a huge damage to agricultural economic growth. The pathogen can produce the survival structure as sclerotia, which are difficult to control [1]. Chemical fungicides are commonly used in current agriculture to control the disease in plant. However, using chemical control is toxic to human and environment. In addition, the pathogen has showed resistance to fungicide. These results cause scientists to search for an alternative method to control the disease in plant, which is less chemical uses and more environmental friendliness [2] [3].

Biological control is an alternative way to control the disease. Some rhizobacteria have been used to control S. rolfsii such as Pseudomonas sp., Burkholderia cepacia, Bacillus subtilis [4]. This study was conducted to isolating and testing rhizosphere bacteria for inhibition growth of mycelium of S. rolfsii under laboratory.

II. MATERIAL AND METHODS

A. Fungal Pathogen

Sclerotium rolfsii was obtained from Section of Plant Pathology, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University. The culture were kept at 4 °C on Potato Dextrose Agar (PDA) slant until use.

B. Isolation of Bacteria

Soil samples and tomato roots were collected from 7 provinces (Kalasin, Khon Kaen, Nong Bua Lumphu, Nong Khai, Ubon Ratchathani, Udorn thani and Yasothon) in northeastern region of Thailand. The soil samples were serially dilution and spreaded on Nutrient agar (NA) plates. The plates were incubated for 24-48 h. Different colonies growing on plates were picked. The selected colonies were restreaked as purified cultures and kept at 4 °C on NA slants for further study.

Tomato root samples were sterilized on the surface, described by [5] [6]. The roots were washed with tap water, cut (10 cm) and submerged for 3 min in 70% ethanol follow by 3% sodium hypochlorite. Each segment was rinsed 2 times in sterile distilled water. Then, the segments were ground in sterile mortar. The bacterial suspension was performed as serial dilution and spreaded on NA plates. The plates were incubated at 30°C. After 48 h different colonies were selected. The purify bacteria were stored on NA slant at 4 °C for further study.

C. In Vitro Antagonism Test by Dual Culture

S. rolfsii was cultured on PDA at 30 ±2°C for 3 days. Then, 8 mm diameter disc was placed onto the center of fresh PDA plates. The bacterial isolate was streaked on the periphery, 2 cm from the edge of the plate (diameter of petri-dish 90 mm). The petri dishes were incubated at 30 ±2°C for 5 days and fungal colony diameters were measured. The control was S. rolfsii on PDA plate without bacteria. The experiment was conducted in three replicates. Percent inhibition was calculated using the following formula:

\[ \text{Inhibition Percentage (\%) = } \frac{A_1 - A_2}{A_1} \times 100, \text{ where } A_1 \text{ is colony diameter in the control, and } A_2 \text{ is colony diameter in the dual culture [7].} \]
The data were analyzed by standard analysis of variance (ANOVA). The mean values between treatments were compared by Duncan’s Multiple Range Test (DMRT) at \( p \leq 0.05 \) level of significance using software SPSS Windows version 19.

D. Test of Culture Filtrate on Mycelial Growth of Fungal Pathogen

The antagonistic bacteria were cultured in 100 ml of Nutrient broth (NB). The cultures were incubated on a rotary shaker (150 rpm) at room temperature for 48 h. The suspension of bacteria was centrifuged at 13,000 rpm for 30 min. The supernatant was filtered through a 0.45 \( \mu m \) pore-size filter as culture filtrate [8]. A mycelial disc (8 mm diameter) of \( S. \) rolfsii was transferred to a sterile petri dish (diameter = 5 cm) containing 8 ml of the culture filtrate. The plates were incubated at 30 ± 2°C for 6 days. The control plate was \( S. \) rolfsii mycelial disc in fresh NB (applied as described by [9]). Mycelial growth was compared using a scale from 0-2, where 0 = no mycelial growth, 1 = limited of mycelial growth around disc, and 2 = overgrows of mycelia in liquid medium [10]. The experiment was employed with three replications/isolate.

E. Morphological Characters and Identification of Antagonistic Bacteria

The antagonistic bacteria were characterized for prominent features such as colony morphology and gram stain. Based on molecular characterization, total genomic DNA was extracted. The 16S rDNA was amplified by PCR using the primer UFUL (GCCTAACACATGCAAGTCGA) and URUL (CGTATTACCAGGCTGTCTGG) [11]. The sequence data were analyzed by Mahidol University-Osaka University Collaborative Research Center for Bioscience and Biotechnology (MU-OU:CRC). The phylogenetic tree was constructed by the NJ tree with MEGA version 5.05 software.

### Table I. The Number of Bacteria Isolated from Rhizosphere Soils and Roots of Tomatoes in 7 Provinces from Northeastern Region of Thailand

<table>
<thead>
<tr>
<th>Source of Samples</th>
<th>Root of Tomato</th>
<th>Rhizosphere of Tomato</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of samples</td>
<td>Number of bacteria</td>
<td>Number of samples</td>
</tr>
<tr>
<td>Kalasin</td>
<td>2</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Khon Kaen</td>
<td>10</td>
<td>39</td>
<td>13</td>
</tr>
<tr>
<td>Yasothon</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Nong Khai</td>
<td>2</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Nong Bua-lumphu</td>
<td>8</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>Udon thani</td>
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<td>3</td>
</tr>
<tr>
<td>Ubon</td>
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<td>19</td>
<td>5</td>
</tr>
<tr>
<td>Ratchathani Total</td>
<td>30</td>
<td>111</td>
<td>41</td>
</tr>
</tbody>
</table>

III. RESULTS

A. Isolation of Bacteria

Seventy-one samples were collected from rhizosphere soil and root of tomato in Thailand. Three hundred and twenty-nine bacterial isolates were obtained (Table I).

**Figure 1.** Effect of antagonistic bacteria against \( Sclerotium rolfsii \) by dual culture assay. The numbers 1-11 are isolates of bacteria (1) control, (2) YABA-7, (3) UB3EBa-4, (4) KA1EBa-1, (5) KA1EBA-4, (6) KK7Ba-3, (7) KK7Ba-2, (8) KK9Ba-1, (9) KK1EBa-3, (10) KK11EBa-3 and (11) UD1EBa-2. Means with different letters were significantly different (\( p \leq 0.05 \)) in % growth inhibition (Duncan’s multiple range test).

B. In Vitro Antagonism Test by Dual Culture

The isolated bacteria were tested for inhibition mycelial growth of \( S. \) rolfsii by dual culture method on PDA plates. The results showed that thirteen isolates, YABA-7, UB3EBa-4, KA1EBa-1, KA1EBA-4, KK7Ba-2, KK7Ba-3, KK9Ba-1, KK1EBa-3, KK11EBa-3, and UD1EBa-2 could reduce mycelial growth in ranges 45.00- 60.00 % with statistical significance (\( p \leq 0.05 \)) (Fig. 1). The isolate KK11EBa-3 showed the highest inhibition at 60.00% (Fig. 2).

**Figure 2.** Bacterial isolates against \( Sclerotium rolfsii \) by dual culture. (A) only \( S. \) rolfsii, (B) KK1EBa-3, (C) KK11EBa-3, (D) UD1EBa-2.
C. Effect of Culture Filtrate on Mycelial Growth of Fungal Pathogen

The culture filtrate of bacteria was tested for mycelial growth inhibition. The result revealed that the culture filtrate of three bacterial isolates (UD1EBa-2, KK1EBa-3, KK11EBa-3) could completely control the growth of S. rolfsii (no mycelial growth) after 6 days incubation at 30 ± 2 °C when compared with control (Fig. 3).

Figure 3. Inhibition of mycelial growth of Sclerotium rolfsii by culture filtrate bacteria. (A) only S.rolfsii, (B) KK1EBa-3 (C) KK11EBa-3, (D) UD1EBa-2

D. Morphological Characters and Identification of Antagonistic Bacteria

The effective antagonistic bacterium KK11EBa-3 was characterized to be rod shape, non-spore forming and negative gram (Fig. 4). The bacterium was cultured on NA for 48 h. The colony was circular, smooth, translucent, flat elevation. Based on molecular characterization, it was identified as Pseudomonas aeruginosa (Fig. 5).

IV. DISCUSSION

Three hundred and twenty-nine isolates of bacteria were tested for their ability to inhibit the growth of Sclerotium rolfsii using dual test on Potato Dextrose Agar (PAD). Three isolates (KK1EBa-3, KK11EBa-3, and UD1EBa-2) showed strong inhibition with 51.25%, 56.25%, and 60.00% respectively. The most effective isolate (KK11EBa-3) was identified as Pseudomonas aeruginosa by molecular characterization. Similarly, Kishore et al. [12] reported that P. aeruginosa could inhibit mycelial growth of S. rolfsii up to 74 % using dual culture. This research is the first step to use an effective microorganism against S. rolfsii. Shoda [13] described that sometimes the results from in vitro, in greenhouse and in fields showed no correlation. Therefore, a plant test should be used in experiments to confirm efficiency of biocontrol agents. In a further experiment, the effective isolate is tested in controlling tomato stem rot and collar rot in greenhouse and in fields.

ACKNOWLEDGMENTS

This work was supported by grants from the Human Resource Development in Science Project (Science Achievement Scholarship of Thailand, SAST) and Financial support for this research was provided by the General Supporting Research Fund of Khon Kaen University, Thailand.

REFERENCES

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